THE ROLE OF SOIL - MICROORGANISMS IN KARST CORROSION

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Summary

Soils in karstic areas are easily damaged by human activity and hard to sustain. Therefore, it is very important to investigate the processes in these soils from the point of view of conservation of karst. The life-activity of biological-microbiological communities, as principal sources of CO_2 and various acids in the soils, play an important role in karst corrosion, though the main aggressive sources of soils have not been described and measured exactly. This paper describes new laboratory analyses of the production of material aggressive to $CaCO_3$ by selected soil microbes. The process of pH change by microorganisms and the amount of $CaCO_3$ dissolved by selected microorganism strains were measured under „optimal" conditions. Data were obtained on the difference between CO_2 and other chemicals, and the production of aggressive agents by various microorganism groups. At the same time, the most important microbial strains, their behaviour, and the processes controlled by them under different environmental conditions were also examined. Furthermore, the quantity of microorganisms was determined in soil samples gathered on the Aggtelek Karst in Hungary.

Introduction

„Microorganisms are dangerous - they make one ill" - this is probably the image most people have of microorganisms. Really, what does it mean microorganisms from the aspect of karst research? These problems are relatively difficult to investigate because they possess an interdisciplinary relation, but might help to explain that in what degree the soil-microorganisms play an important role in the processes of the karst corrosion. Today, researchers of the role of types of soil cover on a limestone surface attach a great importance to their direct influence on the karst relief evolution. The complex soil effect is due to the joint influence of many factors (soil permeability, evapotranspiration, microclimate, soil aeration, soil life, and others), which together control the amount and aggressivity of karst water (*Kevei-Bárány and Zámbó, 1985-86; Zámbó, 1991, 1993)*. One component is the

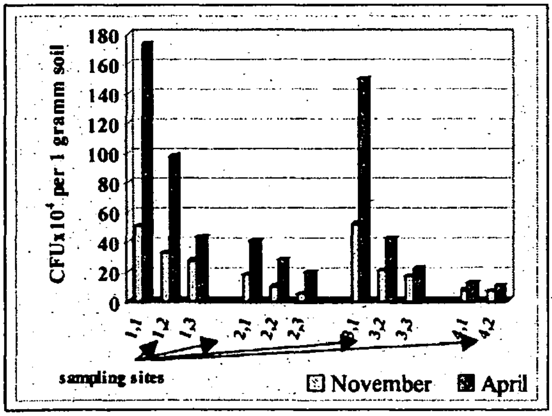


Fig. 1 Colony Forming Units of aerobic and facultatively anaerobic bacterial communities of the soil samples taken from

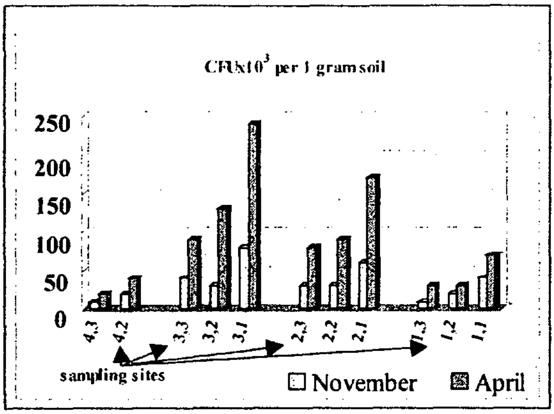


Fig. 2 Colony Forming Units of microfungi communities of the soil samples taken from Béke-dolina

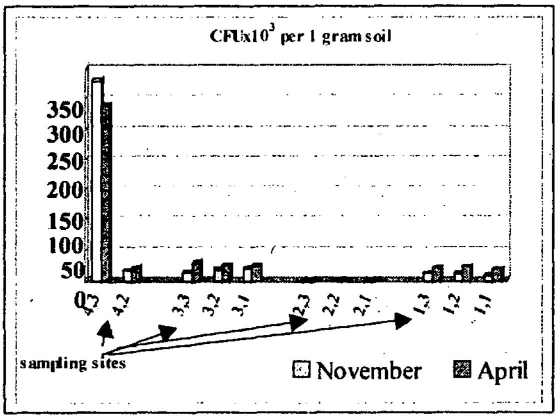


Fig. 3 Colony Forming Units of anaerobic bacterial communities of the soil samples taken from Béke-dolina

amount of CO_2, which is originated from the soil biological activity. The major part of produced CO_2 amount is attributed to the activity namely aerobic or anaerobic respiration or fermentation of soil-microorganisms - such as bacteria and microfungi. Moreover any acids, whether inorganic or any of a variety of organic acids produced by subsurface microbes in consumption of organic matter for energy production, would also be very corrosive, depending on the intensity of metabolism of microbes (*Ehrlich* 1998; *Jakucs at al. 1983*; *Lovley and Chapelle, 1995).* The regular renewing of aggressive matter production by microorganisms alters the chemical composition of the soil atmosphere, and later that of the infiltrating waters *(Killham 1994).* Therefore, the microbial life-activity indirectly becomes the principal affecting factor of karst corrosion processes and determining the circumstances and rate of the limestone corrosion by microbes is very interesting and complex problem in the karst research in Hungary, too.The aim of the present paper is to give more information about microbiological communities in the soil of limestone areas and their role in the processes of limestone corrosion.

Methods and research strategy

There are several methods to able to investigate the solution of limestone (karst corrosion) by soil-microorganisms' s activity. The stages of present research strategy were the following:

- Determination of the sampling sites and methods,
- Selecting some dominant soil types covered the Hungarian karst areas and collecting the soil samples (*Szegi 1979)* from the following soil types, in

Béke-doline of Aggtelek Karst, which are also common in other karst regions in Hungary.

1. Soil type of terra rossa-like (*Zámbó 1993)* of more than 1 m thickness, common in karstic depressions
2. Rendzina (T_2) of 0.3 m thickness, common on karst slopes and uplifted surfaces
3. Soil type of terra rossa-like(T_1)of 2 to 3 m thickness, common on karst slopes and the valley floors
4. Doline fills of more than 5 m thickness

In the cases of „1", „2" and „3" the soil levels of 0-10 cm; 10-20 cm and 20-30 cm were examined and in the 4th case, the levels of 270 cm and 750 *cm (Zámbó and Darabos 1993*).

- Examination of the number and the topographical distribution of the main groups of microbes living in the „karst soil" and identification of the isolated microorganisms (microbiological methods */Bergey' 1986; Collins 1967; Covan, and Steel 1965; Szabó 1974; 1986/*)
- Carrying out some new laboratory model analysis using some selected microbes (The methods of laboratory analysis were invested by the author.)

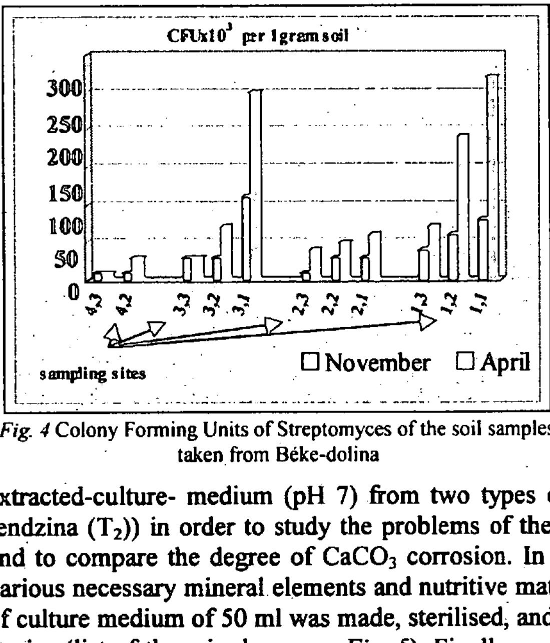
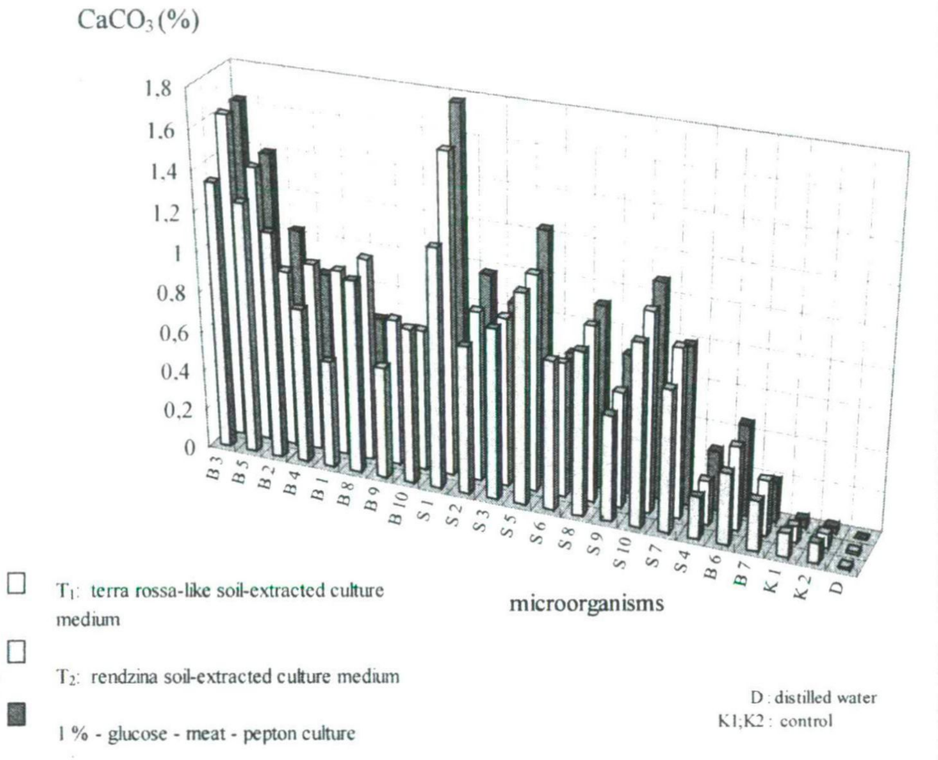


Fig. 4 Colony Forming Units of Streptomyces of the soil samples taken from Béke-dolina

In the laboratory model analysis, the CO_2 production of the individual strains of microbes was calculated in the artificial environment. Thus, significant data could be obtained for the difference of CO_2 production between each of the selected strains under the different circumstances. For the purposes of this, 50g of cleaned limestone rubble of 3.5 to 5 mm diameter was put into Erlenmeyer-retort and the whole system was sterilised in the autoclave. In the other retorts, we used 1 %-glucose-culture-medium (pH 7) and soil-extracted-culture- medium (pH 7) from two types of soils (soil like terra rossa (T_1) and rendzina (T_2)) in order to study the problems of the nutritional requirements of microbes, and to compare the degree of $CaCO_3$ corrosion. In the soil-extracted culture medium the various necessary mineral elements and nutritive matters were available for microbes. Each of culture medium of 50 ml was made, sterilised, and inoculated by clasper unit of different strains (list of them is shown on *Fig. 5*). Finally, we poured the inoculated medium on the limestone rubble in the sterile conditions. After all the above, the retorts were incubated up to a definite time in laboratory temperature (21°C). At the end of incubation period, we measured the pH-values of culture mediums taken-out and the weight of cleaned limestone rubble. As control tests, we made the same amounts of limestone dissolve in distilled water (D) and in microorganism-free-medium (K).

In the following investigation, we used special culture medium (Czapek-medium of pH 5) which is an ideal environment for microfungi and in which the growing and activity proved to be bigger than that of other microbes (*Szegi 1973*).



B1	B. megaterium var. 1.		B6	M. luteus		S1	S. cellulose		S6	S. gelaticus	
B2	B. cereus var. 2.		B7	B. megaterium var. 7.		S2	S. atroolivaceus		S7	S. diastaticus var.	
B3	B. megaterium var. 4.		B8	B. megaterium var. 8.		S3	S. diastaticus		S8	S. exfoliatus	
B4	M. kristinae		B9	B. cereus		S4	S. spadicis		S9	S. atroolivaceus var.	
B5	B. mycoides var.		B10	B. mycoides		S5	S. anulatus		S10	S. actuosus	

Fig. 5 $CaCO_3$-corrosion by microorganisms in the different types of the culture medium (B: Bacterium, M: Micrococcus, S: Streptomyces)

Result and discussion

1. Analysing the CFU number and the topographical distribution of the main groups of microbes (*Fig. 1, 2, 3, 4*) in autumn and in spring periods, usually large population of microbes was found in each spring-soil samples. The quantity of *Bacterium* (and *Sterptomyces* – a kind of *Bacterium*) strains proved to be bigger than that of microfungi. Except of the doline's depth, the major part of the microbes was the aerobe bacteria at each of the sites. Usually, the number of microbes decreased following the

depth. The great number of anaerobe-microbes in the deepest zones, which are hardly ventilated, is significant.

2. The most common 10 *Bacterium* and *Streptomyces* strains, which were found at all the sample sites, were isolated, selected and finally identificated (*Fig. 5*) in order to be used in the further laboratory analysis.

3.a. Shown by Figure 5, the amount of dissolved $CaCO_3$ was different depending on the strains, because they produce different amounts of $CaCO_3$-aggressive materials (CO_2 and carbonic acids). The dissolution of $CaCO_3$ was bigger in the 1 % - glucose - meat - culture medium than in both of the soil-extracted-culture mediums. This happened because the organic carbon is the major constituent of the food supply and it means a strong stimulation for the microbial activity. Anyway, the significant loss of limestone in the soil-extracted-culture mediums leads us to suggest that these soil types represent favourable conditions for microorganisms and these soil types for karst corrosion. The solution (corrosion) in the terra rossa-like (T_1) soil-extracted culture medium was usually more significant than the solution in the rendzina (T_2) soil-extracted culture medium. In the cases of the control tests, and the solution impact of the nutrient solution on the limestone was negligible. At the same times the pH changing was measured in every case. If the amount of $CaCO_3$ was considerable, then the pH-change, change between the starting pH-value and the end of the process pH-value, was also significant. On the other hand, when the amount of $CaCO_3$ dissolved during the process was not very big, then the pH-value also differed less at the end of the process from the starting value. This can be explained with the influence of the microorganisms present at the tests and their metabolic products (CO_2 acid and other acids) on the $CaCO_3$.

The various strains adapted to the environments in different ways, thus they produced aggressive matters differently as well, therefore the effect, and the observed amount of dissolved $CaCO_3$ differed depending on the various strains of microorganisms. Results of the test it can be concluded, that the pH-value and the composition of the nutritive matter of the culture medium is influencing a great deal the activity of microorganisms.

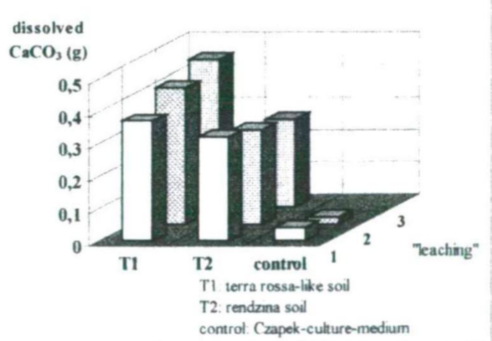


Fig. 6 $CaCO_3$ -corrosion caused by terra rossa-like (T1) and rendzina (T2) soil types in the Czapek - culture- medium (pH 5)

3.b. As the result of this test (*Fig. 6*), the microfungi have a significant corrosion effect, too. According to the result of this test, it can suggest that, the low pH-value (4 or 5) of condition existed in the „karst soil" are made the species-composition of original micro-organism community changed, and can reduce the microorganisms activity producing CO_2. The changes in the easy-to-wound and hard-to-sustain soils covering the karstic areas are caused mainly by human activity. Therefore, these results are very important from the point of view of conservation of karst.

Conclusion

It was clearly shown by the different tests that the life-activity of microbial communities, as principal sources of CO_2 and various acids in the soils, play an important role in karst corrosion. The various ecological factors (pH-value, the amount of acids, microelements and ions etc.) have a very important and determining role for the activity of microorganisms. Under the optimal conditions the activity of microorganisms is usually a decisive importance in the production of aggressive material by karst soil. In natural conditions, of course, we have to consider the complex effects of karst soil types, and that the microorganism strains are not acting alone, but the result of their joined actions are reflected in the process of the karst corrosion and in the evolution of karst areas.

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